"High-Resolution, Ultra-Sensitive Magnetic Imaging Using an Ensemble of Nitrogen-Vacancy (NV) Centers in Diamond"

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Name of Contractor:	Quantum Diamond Technologies Inc.
Principal Investigator, Project Scientist, or Engineer:	Colin Connolly
Business Address:	4 Brattle Street, Suite 209, Cambridge, MA 02138
Phone Number:	(617) 320-4105

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Introduction

Magnetic field imaging using nitrogen-vacancy (NV) centers in diamond has a number of compelling life sciences applications, owing to useful characteristics such as high sensitivity down to nanometer-scale resolution, inherent biocompatibility, no need for electrical sensor contact, and highly-parallel detection of many pixels simultaneously. Quantum Diamond Technologies Inc. (QTDI) has focused primarily in Phase I on one target application and modality: high-throughput, high-sensitivity detection of rare cells and biomarkers. QDTI made significant progress toward this goal in the First Quarter, as reported here.

Identification of the target application

Phase I effort commenced with market research. QDTI investigated which commercial applications of magnetic field imaging with diamond are the most compelling targets, including an assessment of the technological requirements for each and whether significant progress was achievable in Phase I. Given the wide array of existing and more mature magnetometer technologies, several of which have shown impressive recent advancement in sensitivity and/or resolution, it was critical that potential applications of NV magnetometry could leverage its competitive advantages. In particular, diamond sensors are set apart by the ability to sense magnetic fields under ambient biological conditions with sub-micron resolution over millions of simultaneous channels, which enables a number of novel magnetic sensing modalities. These are most naturally applied to biomedical applications and life sciences research, for which there exist large, growing markets with significant unmet needs.

While there are many natural biomagnetic signals (including those from hemoglobin in red blood cells, nuclear magnetic moments, and action potentials in nerves and individual neurons), much stronger signals can be obtained by tagging cells or structures with magnetic nanoparticles (MNPs). MNPs functionalized with antibodies or other targeted vectors are a powerful tool for highly specific magnetic labeling, and have for some time been used to magnetically tag a wide range of proteins, cells, and structures [1].

Among several prospective applications, QDTI has identified one in particular as the focus of Phase I: an assay for the detection of rare cells and biomarkers tagged with MNPs. Unlike labeling with fluorescent dyes or quantum dots, detection of magnetic tags occurs over a very low magnetic natural background and hence can be highly sensitive. In addition, the magnetic field of the MNP tags permeates through contaminants, so that minimal sample preparation is necessary.

Of particular interest is enumeration of circulating tumor cells (CTCs) in the bloodstream, which

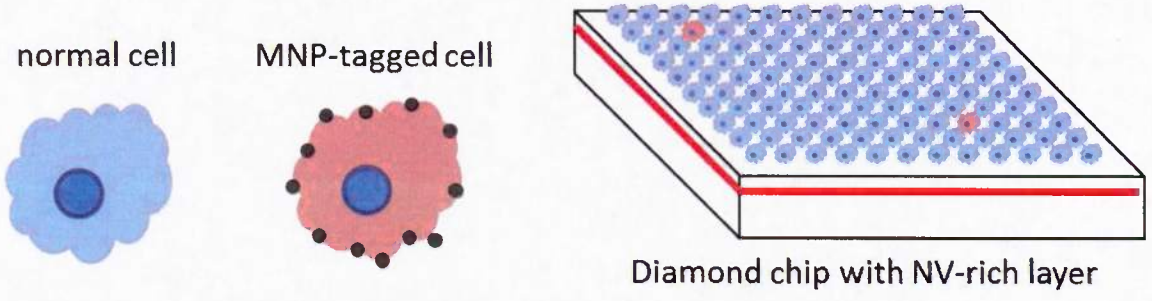

Figure 1: Schematic of the concept of a rare cell detection platform based upon a diamond NV magnetic sensor. NV center fluorescence from a wide field of view is collected to determine the magnetic field below each cell. Hence cells tagged with magnetic nanoparticles (MNPs) can be detected while scanning many cells at once.

are correlated with several cancers and especially with metastasis [2]. CTCs occur at part-per-billion concentrations among normal blood cells, presenting a formidable challenge for accurate analysis. A number of technologies have been employed to detect CTCs above this background, but there remains no flexible gold standard platform for high-sensitivity, high-throughput CTC enumeration [3]. A diamond-based magnetic imaging device has the capacity to probe millions of cells simultaneously in a wide field of view (Figure 1), and to do so with single-cell sensitivity. In addition, simplified sample preparation can avoid losing target cells (current FDA-approved technologies fail to detect the great majority of CTCs [4, 5]) and allow for total assay time for patient samples to be well under an hour.

Feasibility studies

In order to validate the NV diamond magnetic imaging technology for rare cell detection, QDTI has begun a series of feasibility studies. Around the world, NV diamond magnetometry has in the last two years been employed toward related biological goals, for example to measure the magnetic moment of individual magnetotactic bacteria (which manufacture their own MNP) [6] and to image the cell membrane of a HeLa cell (a standard cancerous human cell line) [7]. No efforts have yet demonstrated detection of cells tagged with MNPs, nor achieved the wide field of view necessary for rapid-throughput detection.

QDTI's First Quarter technical objectives included studies of the sensitivity of NV diamond magnetometry to the DC magnetic field produced by superparamagnetic MNPs inside or on the surface of human cells. Superparamagnetism refers to the case when the time scale for magnetic relaxation of the MNP is much shorter than the measurement time, such that the MNP magnetization can be taken as being always in equilibrium [1]. Under such conditions, the MNPs act as paramagnetic particles and align rapidly with a modest external applied field, without hysteresis or significant zero-field magnetization. This is a powerful effect for generating much larger magnetic fields than would be achieved with thermal polarization of non-interacting electronic spins.

To facilitate rapid validation of diamond as a MNP-tagged cell detector, and to explore other potential biological and clinical applications, QDTI in Phase I developed collaborations with researchers at the Center for Systems Biology at Massachusetts General Hospital (MGH) in Boston. Prof. Hakho Lee, in particular, is an expert in the fabrication and use of surface-functionalized MNPs. Prof. Lee has been involved in pioneering efforts for detecting magnetically-tagged cells using NMR and Hall effect sensors [4, 8, 9]. QDTI has partnered with Prof. Lee's lab to obtain MNP-tagged cells. In addition, QDTI has collaborated with researchers in the lab of Ronald Walsworth at Harvard University in order to characterize the capability of an existing NV diamond magnetic imaging apparatus [6, 10] for detection of tagged cells.

The first study used human macrophage cells internally loaded with MNPs. Macrophages are immune system cells that remove foreign material and the detritus of dead cells from the body. When incubated in a suspension of free MNPs, macrophages will internalize a large quantity of particles [11]. This nonspecific macrophage loading does not require conjugation of antibodies to the MNPs, and hence is a rapid and flexible test platform. In addition, however, macrophage tagging has clinical utility that may lead to an additional *in vivo* assay target, arising from correlation of the local macrophage population with inflammation and tissue damage.

Magnetic imaging apparatus and procedure

A schematic of the magnetic imaging apparatus is shown in Figure 2. Desiccated MNP-tagged cells lay on the surface of a diamond chip, where a thin (about 7 nm thickness) layer of NV centers has been implanted about 20 nm below the surface at a density of about 3×10^{11} cm^{-2}. A region of the NV layer is illuminated from below by a 532-nm excitation laser. Total internal reflection of the pump laser

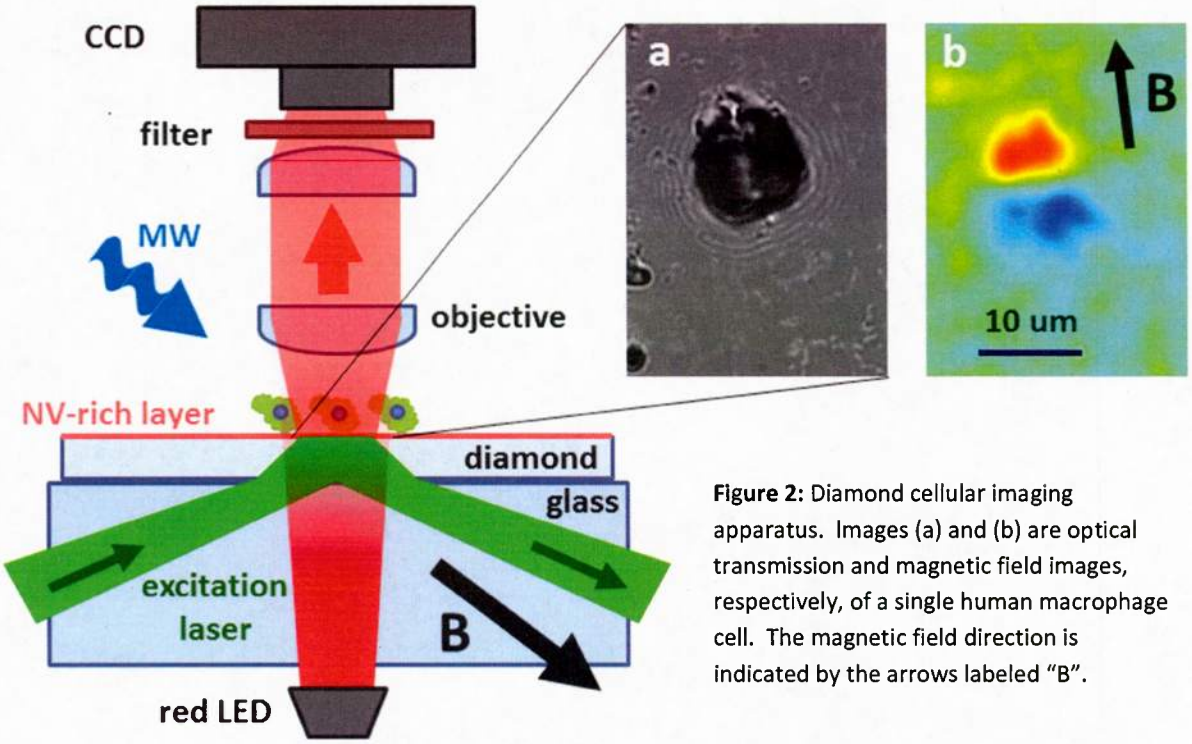

Figure 2: Diamond cellular imaging apparatus. Images (a) and (b) are optical transmission and magnetic field images, respectively, of a single human macrophage cell. The magnetic field direction is indicated by the arrows labeled "B".

at the surface prevents illumination of the cells, which can cause heating (this approach is not required, however, for a detection assay that does not need to preserve the cells). Red fluorescence from the NV centers is collected by an objective lens and imaged onto a CCD camera through an optical filter that blocks scattered excitation light. Alternately, a red LED below the diamond can illuminate the cells for optical transmission imaging. Microwaves near 3 GHz radiate from a simple wire loop to drive transitions between magnetic sublevels of the NV ground state, for electron spin resonance (ESR) measurements. Finally, a static magnetic bias field of about 150 G is applied, aligned to one of the four diamond crystal axes, which includes components both normal and parallel to the diamond surface.

The images labeled (a) and (b) in Figure 2 are optical transmission and magnetic images, respectively, of a single MNP-loaded human macrophage cell, with the same field of view. The MNPs in the cell are polarized by the magnetic field to produce a cumulative dipole-like field. A series of 25 camera exposures are taken under identical laser excitation while varying the microwave frequency across the Zeeman-shifted NV ESR resonance. The field distortion produced from the MNPs locally shifts the resonance by approximately 2.8 MHz/G. The set of 25 fluorescence images constitutes an ESR measurement at each pixel. Fitting separately at each pixel gives the resonance center location, and hence the local magnetic field projection onto the NV axis. Only one quarter of the NV centers participate in the measurement, since only these have the NV axis aligned with the magnetic field.

The magnetic field image (Figure 2(b)) shows a magnetic field distortion of approximately ±4 μT. The imaging system used for the measurement has submicron resolution, as shown in the optical transmission image (Figure 2(a)). The magnetic image has been filtered for clarity, as no sub-cellular structure is expected nor is apparent. Fundamentally, detection of a single cell requires only one bit of information to be extracted (tagged or not tagged). Hence this image is much more detailed than necessary and there is significant overhead for sacrificing resolution and sensitivity in order to increase the field of view and imaging speed, both critical to assay throughput.

Insight from feasibility studies

First-Quarter feasibility studies have produced a number of findings that will be used to optimize the design of a diamond-based rare cell detector. The insight that has been gained thus far also applies more broadly to other diamond magnetometry applications and potential assay targets.

The successful magnetic imaging of a single MNP-loaded human cell demonstrates the fundamental capacity for detection of magnetically-tagged targets. Uncertainty in the quantity of MNPs internalized by macrophages makes it difficult to draw strong conclusions for detection of immunologically-tagged cells (for which, in contrast, MNP loading is strongly correlated with antigen expression). However, there are two reasons to believe that the magnetic field will be stronger from cells tagged with antibody-conjugated MNPs than from macrophages. First, bulk magnetization experiments performed after incubating macrophages with varied suspensions of MNPs observe MNP loading at levels similar to what has been demonstrated with immunological tagging [4, 11]. Second, the MNPs attached to cells with antibodies are expressed on the cell surface closer to the diamond rather than inside the cell, and hence can produce a significantly higher field in the diamond due to the strong $1/d^3$ dependence of the field on the separation d. Therefore we expect similar or better signals to be measured for these cells.

Measurements were also made of the impact on DC magnetic sensing with diamond of crystal strain. Strain from dislocations during crystal growth, surface polishing, temperature gradients, or other sources also produces life shifts that can be misinterpreted as magnetic field [12]. Figure 3(a) shows an example of such apparent line shifts due to dislocation strain, which are especially problematic because they appear similar to magnetic dipoles and have length scales similar to those of biological cells.

Strain shifts can be mitigated in several ways, including: (1) by employing low-strain diamond material for which any shifts are minimal compared to Zeeman shifts from the sample; (2) subtracting normalization images taken with a clean diamond surface, assuming the strain shifts are static; or (3) measuring the frequency difference between ESR resonances coupling the $m = 0$ state to the $m = \pm 1$ states (see level diagram in Figure 3(d)), which eliminates common-mode longitudinal strain shifts while

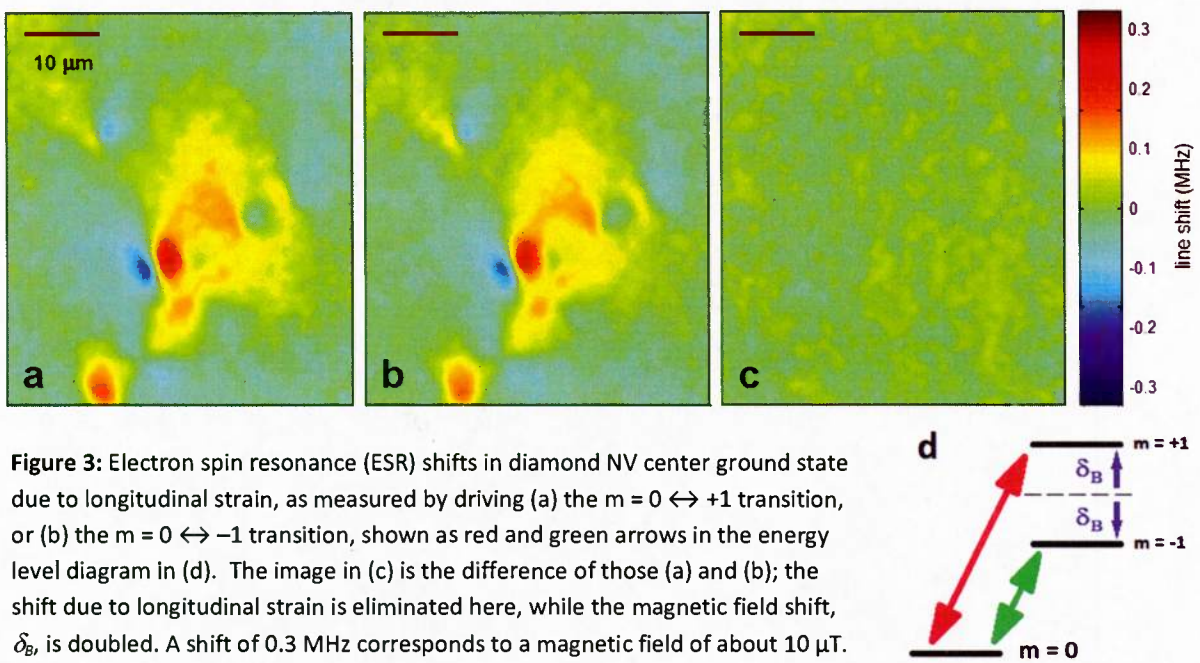

Figure 3: Electron spin resonance (ESR) shifts in diamond NV center ground state due to longitudinal strain, as measured by driving (a) the m = 0 ↔ +1 transition, or (b) the m = 0 ↔ –1 transition, shown as red and green arrows in the energy level diagram in (d). The image in (c) is the difference of those (a) and (b); the shift due to longitudinal strain is eliminated here, while the magnetic field shift, δ_B, is doubled. A shift of 0.3 MHz corresponds to a magnetic field of about 10 µT.

doubling Zeeman shifts. While method (1) is ultimately the most robust, methods (2) and (3) may be more practical given available diamond material. Method (3), in particular, provides strong rejection of first-order longitudinal strain shifts (Figure 3). Transverse strain shifts are themselves suppressed by the ratio of the shift to the Zeeman shift, and hence can be dramatically reduced with modest magnetic fields of 100 G. QDTI is confident that this demonstration of strain shift rejection ensures that a cell detection device can be sufficiently immune to strain effects.

Summary and future outlook

QDTI has made significant progress in the First Quarter of Phase I, including successful completion of the first milestone, magnetic imaging of a single cell tagged with MNPs. Initial feasibility studies have provided useful insight into the signal size, detection threshold, and optimal device design parameters. In the Second Quarter, this validation process will continue with the imaging of immunologically-tagged cancer cells toward the goal of a diamond-based detector for circulating tumor cells (CTCs). In addition, the field of view will be expanded to accommodate imaging many cells simultaneously, toward rapid parallel detection.

In addition to laboratory studies, QDTI has actively explored the market for assays targeting rare cells and biomarkers, including CTC enumeration. This effort includes outreach to clinical end users to understand the technological needs and operational environment for this application. This effort will accelerate in the Second Quarter, fueled in part by supporting data from feasibility studies.

The Phase I milestones represent significant progress toward an ultimate assay goal of processing one million cells/min with single-cell sensitivity, which would allow a diamond-based device to strongly compete with other technologies in the detection of rare cells. Such an assay is clearly achievable with existing technology, given demonstrated sensitivity and the paths identified for scaling up to faster and more parallel cell detection. Furthermore, dramatic improvements could be made in speed, sensitivity, and multifunctionality—for example, by sensing the AC field created by driving the MNPs or by adding optical fluorescence detection as an orthogonal channel to veto weak signals from MNPs nonspecifically bound to leukocytes.

We also note that this rare-cell assay goal represents an important step in validating the utility of diamond-based magnetometry techniques for biological research and clinical applications. This near-term goal will lead to useful technological development and will give diamond NV sensors greater exposure to the life sciences community. This will catalyze new devices and applications to fully utilize the impressive capabilities of this technology, which may include molecular-scale MRI and real-time imaging of neural networks.

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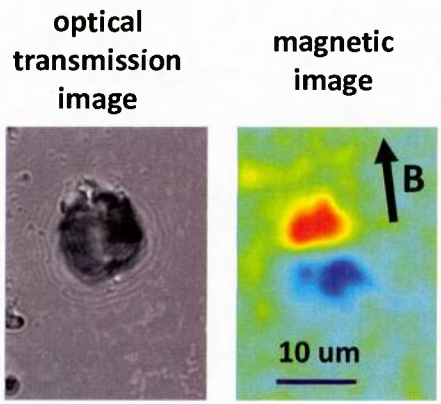
Publications this period

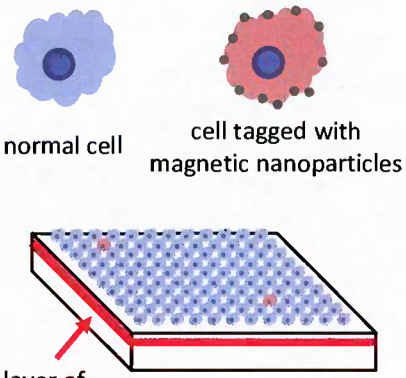
There were no publications sponsored by this contract in this reporting period.

High-resolution, Ultra-sensitive Magnetic Imaging Using an Ensemble of Nitrogen-Vacancy (NV) Centers in Diamond

Magnetic imaging of magnetically-tagged single cells



Macrophage cell loaded with magnetic nanoparticles laying on diamond surface



Target Application: Detection of Rare Cells and Biomarkers

Advantages of diamond sensors:

- High sensitivity
- High resolution
- Room temperature operation
- Biocompatibility
- No electrical sensor contact
- Parallel detection

Natural applications to biomedical assays

Functional magnetic tagging with magnetic nanoparticles can be used to label cells, sub-cellular structures, proteins pathogens, etc. Immunological tagging with antibodies allows for highly-specific labeling of certain rare targets.

Competitive advantage

- Magnetic detection has low background, compared to optical fluorescence.
- Can use unpurified samples (e.g., whole blood) without spoiling detection, enabling rapid sample preparation and faster assay time
- Nanoparticle tags are non-toxic, compatible for *in vivo* application

Clinical Utility and Significance

- An NV diamond rare cell assay could be used for enumeration of **circulating tumor cells (CTCs)** in the bloodstream, at concentrations of a few cells per billion normal blood cells
  - CTCs are indicative of cancer progression and metastasis
  - Existing FDA-approved technologies miss the great majority of CTCs, require hours of assay time
  - **High-throughput** device with **single-cell sensitivity** would provide a dramatic improvement in clinical diagnostic power, and might allow for extensions to low-CTC pathologies such as ovarian cancer.
- Same detection method could be applied to **pathogens**, **proteins**, etc.
- Important step toward wide implementation of diamond NV sensors for biomedical applications.

Phase I Technical Objectives

Quarter 1

- Market research and applications development
- Sensitivity studies with cell tagged with magnetic nanoparticles (MNPs)
- Expand detection to immunologically-tagged cancer cells
- **Milestone**: Map magnetic field of individual human cell tagged with MNPs

Quarter 2

- Expand field of view for parallel detection
- Implement side illumination with edge-polished diamond
- Improve detection rate to minimize sample time
- Explore detection of low-expression cells with $< 10^4$ MNP/cell
- **Milestone**: Simultaneous wide-field magnetic imaging of over 1,000 cells with ~0.1–1% concentration of tagged cells

Company: Quantum Diamond Technologies Inc. **Contact**: Seabron Adamson **Email**: seabron@gmail.com **Phone**: (617) 320-4105